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PRINCIPAL INVESTIGATOR: **James A. Cardelli, Ph.D.**

CONTRACTING ORGANIZATION: **Louisiana State University Health Sciences Center
Shreveport, LA 71130**

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14. ABSTRACT The original hypothesis was that a novel proprietary compound we identified in a screen named LS122, would be a potent inhibitor of the kinase STK36 which had been proposed to play a role in hedgehog signaling. This pathway was been hypothesized to contribute to progression of castrate resistant prostate cancer (CRPCa). The original proposal and statement of work proposed that inhibition of STK36 would prevent nuclear translocation of Gli, the transcription factor important in this signaling pathway. The proposal also described in Task 2 the use of a high content screen to identify additional STK36 inhibitors using an available kit. We initiated these proposed experiments and to our disappointment, LS122 did NOT affect the Hedgehog pathway at all and furthermore, no kit existed that would allow us to screen for additional STK36 inhibitors. Fortunately, we confirmed that LS122 was a potent NF- κ B inhibitor most likely by targeting the kinase RIP2K which is active upstream of NF- κ B. Since NF- κ B is an important signaling pathway implicated in CRPCa progression, <u>our new SOW focusing on this pathways, is still in line with the overarching mission of developing agents to treat CRPCa.</u> Details of our findings will be presented in the final report. As a positive achievement, we were able to execute a patent of LS122 in the U.S. and Europe.					
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INTRODUCTION

Overview of the Annual Progress Report, Justification for a Modified Statement of Work and a Six Month no Cost Extension Request for W81XWH-12-1-0011

Dr. Jill Williams LSU Health was awarded the original DOD grant (title above) but at about the time the grant was to activate, she accepted a job outside the State of Louisiana. Following an exchange of information, the DOD agreed that Dr. James Cardelli, Director of Basic and Translational Research at the Feist Weiller Cancer Center (FWCC) would assume the role of P.I. on this grant and the application was activated on May 15, 2012. The original hypothesis was that a novel proprietary compound we identified in a screen named LS122, would be a potent inhibitor of the kinase STK36 which had been proposed to play a role in hedgehog signaling. This pathway was been hypothesized to contribute to progression of castrate resistant prostate cancer (CRPCa). The original proposal and statement of work proposed that inhibition of STK36 would prevent nuclear translocation of Gli, the transcription factor important in this signaling pathway. The proposal also described in Task 2 the use of a high content screen to identify additional STK36 inhibitors using an available kit. We initiated these proposed experiments and to our disappointment, LS122 did NOT affect the Hedgehog pathway at all and furthermore, no kit existed that would allow us to screen for additional STK36 inhibitors. Fortunately, we confirmed that LS122 was a potent NF κ B inhibitor most likely by targeting the kinase RIP2K which is active upstream of NF- κ B. Since NF- κ B is an important signaling pathway implicated in CRPCa progression, our new SOW focusing on this pathways, is still is in line with the overarching mission of developing agents to treat CRPCa. Our original studies were also slowed by the lack of activity of LS122 and we needed to wait until the PERD Institute in India made more active compound. In Section 1 below, we will summarize our progress (***bold and italicized***) and problems with the original Tasks proposed. In Section 2, we will outline the progress made in a new direction as well as the new SOW along with a timeline for completion. In Section 3, we will present the original budget, the dollar amounts remaining and the new proposed uses of remaining funds in a 6 month no cost extension.

BODY

SECTION 1 THE ORIGINAL SOW

Original Task 1. Establish cell culture models (months 1-4)

1a. Develop 3D culture system; mix CFSE-labeled PCa cells (e.g., LNCaP C4-2, PC-3, PC-3 docetaxel resistant) with normal and cancer-derived prostate stromal cells (e.g., 19I, 33F, or 33Q clones) in QGel hydrogel or alternatively, in liquid co-culture (months 1-3). **1b.** Record phenotypic growth characteristics using microscopic imaging (months 1-3)

Progress: We have developed a procedure to grow prostate cancer cells and stromal fibroblasts in matrigel and we have acquired taxane resistant PC3 and DU145 cell lines (see figure 1). We have begun experiments to determine if LS122 sensitizes tumor cells to taxane treatment. This subtask should be complete in 6 months.

1c. Isolate CD133+/CD44^{high}/AR- stem-like cells from WPE1-NB26 prostate cancer cells (months 2-4)

Progress: We have not been successful in isolating these stem cells yet but will continue with the metastatic cell-lines PC3 and DU145.

Original Task 2. Screen for other STK36 inhibitors and functionally validate hits (months 2-3)

2a. Conduct in vitro biochemical kinase assay: Briefly, incubate ProQuinase STK36 kinase + substrate peptide + ATP then add ADP-Glo reagent (Promega) and measure luminescence on Bio-Tek Synergy 4 plate reader. Determine IC70 as hit criteria (month 2).

2b. Conduct functional screen to validate hits: As in Task 3b below, using the CellomicsVTI high content platform, measure effect of compound hits on Gli nuclear translocation in LNCaP, LNCaP C4-2 cells, PC-3 docetaxel-resistant cells and WPE1-NB26 stem-like cells (months 2-3)/ Additional hits from this screen will be compared with lead candidate compound and those with equivalent or superior activity will be characterized in in vitro assays outlined in Task 3.

Progress: *Only in the last month has this kit been available and since we conclude LS122 does not target Gli translocation to the nucleus or STK36 activity; this SOW along with task 3 below is not relevant. Furthermore, we consider Tasks 2 and 3 ALONE would require 2 years to complete and would not be feasible on this budget.*

Original Task 3. Test compounds [lead compound(s) ± docetaxel (doses varying up and down from 6nM or IC50 value) or olaparib (doses varying up and down from 3µM)] in various in vitro models for viability and gene expression (months 3-7)

3a. Putative anti-Gli effects on PSA and AR: Test compounds in 2D LNCaP and LNCaP C4-2 cells ± R1881 in androgen-depleted (charcoal stripped serum, phenol red-free T media) conditions on PSA, AR, and Shh expression and viability (month 3)

3b. Effects on Gli nuclear translocation, i.e., transcriptional activation: Using the CellomicsVTI high content platform, measure effect of compound alone on Gli nuclear translocation in LNCaP, LNCaP C4-2 cells, PC-3 docetaxel-resistant cells and WPE1-NB26 stem-like cells (month 4)

3c. PARP inhibitor sensitization: Using the CellomicsVTI high content platform, measure PARP levels in LNCaP, LNCaP C4-2, PC-3 docetaxel-resistant cells and WPE1-NB26 stem-like cells treated with compound ± 0.5 µmol/L olaparib for 2 hours before treatment with 10 mmol/L H2O2 (with or without 0.5 µmol/L olaparib) for 10 minutes in the dark. Fluorescence intensity of PARP in each nucleus of at least 500 cells will be compared between conditions.

3d. Cell viability: Test compounds in 3D models (established in 1a) for viability using Cell Titer Blue reagent. Alternatively, if unsuccessful in establishing 3D model, use liquid co-culture cells (months 3-6)

3e. Isolate cells from 3D (or liquid co-culture) models for western blotting and qRT-PCR (month 3-6)

3f. Gene expression downstream of STK36 (Gli activation) inhibition studies: Conduct western blotting and qRT-PCR for expression of Gli and Gli targets, including Ptch1, FoxL1, SNAIL, TWIST and Bcl2, CD133, CD44, and osteonectin (months 4-7)

The expected results in these experiments are that we will see STK36 inhibition to block Gli activation and translocation to the nucleus, decrease cellular viability, increase PARP inhibitor activity, reverse docetaxel-resistance, increase docetaxel-sensitivity in sensitive cells, and decrease expression of at least a subset of downstream Gli targets, including PSA. Cells were chosen for their response to androgens (LNCaP and derivatives, docetaxel resistance (PC-3 cells-docetaxel resistant) and their previously characterized “stem-like” population (CD133+/CD44high/AR- WPE1-NB26).

Progress: Since Gli is NOT a target for LS122, we have not attempted this task. Instead we have replaced this task with a new task 2 listed below which characterizes the effect of LS122 on the NFKB pathway and the role of RIP2K. In addition, the originally listed tasks 2 -4 are way too ambitious. It would take a year alone to develop new lead compounds and an additional year to test them in the animal model described below.

Original Task 4. Mouse study to determine in vivo effects of lead compound* ± docetaxel and ± olaparib (months 7-9)

This study has 6 groups of 10 mice each, including untreated, docetaxel, olaparib, compound, compound + docetaxel, compound + olaparib as noted below with an expected duration of 45 days.

4a. Culture LNCaP C4-2 luc and stromal cells (see 1a) for subcutaneous implantation into flanks of male 6-8 week old SCID/bg mice (month 7)

4b. Inject 1:1 mixture of 1x10⁶ each type in hydrogel/T media into both flanks; begin treatment lead compound by daily oral gavage at 50 mg/kg, ± continuous dose docetaxel pellet at 8mg/kg/week and ± daily oral gavage of olaparib at 100mg/kg/day (month 8-10)

The lead compound will be tested for proof-of-principle in this initial animal study. Lead compound status will be determined by in vitro results in Task 3. We expect to see a decrease in tumorigenicity, doubling time and final tumor volume. Blood samples will be collected at initiation, midpoint, and at necropsy for PSA analysis. Tumor tissues will be harvested at study end and a portion flash frozen for RNA and protein analysis and a portion formalin fixed for immunohistochemical analysis of proliferation, apoptosis, and Gli target expression as described in 3F.

Progress: Again, this task is not relevant since the Gli pathway does not seem to be impacted by LS122. Instead, we have performed animal experiments supported by our institution that reveal that LS122 slows the growth of PC3 and other aggressively growing prostate tumor cells, as well as impacting metastasis without demonstrable toxicity. We believe this is via inhibition of the NFKB pathway. Therefore, animal studies relevant to this proposal and supported by intramural funds have been performed, and the requested DOD money allocated for this task is proposed to be rolled into supplies.

Original Task 5. Analyze data and write reports (months 10-12)

A manuscript describing the results listed above is in preparation for submission this summer.

SECTION 2 THE NEW SOW

The following section contains a rationale for the new studies, a new SOW combining old tasks with new ones based on preliminary experiments, and a progress report for this new direction. Our overall mission has not changed; only the therapeutic target has.

Rationale for a new SOW:

In the last few years we have seen an increase in new FDA-approved drugs for prostate cancer (CaP), more new molecular targets, and more hope for survival benefit than in the whole decade before. Despite this progress, advanced CaP therapy remains an underserved area of research and none of these newly approved drugs have shown long-term survival benefits. Death and suffering from prostate cancer is virtually all due to progressive, therapy-resistant disease and metastatic prostate cancer is the

deadly form, particularly when it goes to the bone. Our ultimate goal is to provide: 1) a new drug for advanced, metastatic prostate cancer patients that has efficacy to prevent the progression of prostate cancer to hormone and chemotherapy resistance, 2) to limit dissemination of cells to visceral tissues and the bone, and 3) to treat those cells that reach the bone microenvironment.

It had been proposed in the original application, that LS122 would target the Hedgehog pathway which contributes to CRPCa progression. However, our preliminary experiments did not support this hypothesis. Therefore we investigated another pathway important in PCRPCa. In a set of preliminary pre-clinical studies presented below, we have found that the novel small molecule (LS122) to have low toxicity and high efficacy as an anti-cancer and anti-osteogenic molecule most likely by targeting an upstream component (RIP2K) of the NF- κ B pathway. LS122 was discovered in collaboration with the PERD Institute and is classified as a thiophene. The position in the molecule of the substitutions and the substitutions themselves are novel and designed to reduce cardiotoxicity seen in similar drugs of this class, while increasing specificity and efficacy. LS122 was rationally designed as an anti-inflammatory and to protect against the cardiotoxicity associated with FDA-approved drugs in the same drug class. A patent has been filed and executed.

There are several reasons why LS122 is a desirable drug for clinical development: 1) it has potential as a sensitizer to standard of care chemotherapeutics, 2) its kinase inhibitory activity against a small number of important and unique kinases for prostate cancer, 3) the tissue distribution of its targets relevant to human prostate cancer progression, and 4) the low toxicity and high efficacy LS122 demonstrated in preliminary studies. In addition, LS122 is a quinazolinone, which as a class, has been described to re-sensitize resistant cells to chemotherapeutics, particularly taxanes [1]. RIPK2 is overexpressed in adaptive immunity, inflammation and cancer; particularly noteworthy is its overexpression in osteoblasts [2] and our observation of overexpression, as well as phosphorylation, in the PC-3 and LNCaP derivative C4-2B4 prostate cancer cells (unpublished data). RIPK2 is initiated by stimulation of the TNF- α receptor, a common occurrence in human CaP that is linked to progression to castrate-resistance as well as bone metastases; the clinical utility of TNF- α inhibitors for prostate cancer has been suggested for that reason [3-6].

NF- κ B is a downstream target of RIPK2 that is also integral to both castrate-resistant progression and bone metastasis [7-12]. LS122 has shown both direct and indirect anti-NF- κ B activities (see data below) and as such may be particularly useful in controlling functional changes downstream of NF- κ B as well. LS122 has a limited number of kinase targets and therefore potentially fewer side-effects and off-target effects; problems associated with current FDA-approved drugs of this class. We chose to characterize LS122 and prostate cancer because NF- κ B is a key regulator of prostate cancer progression and metastasis, particularly in its role in bone metastasis. LS122 is a new chemical entity with novel substitutions and a novel mechanism of action. LS122 is in a drug class that has historically acted to sensitize chemo-resistant cells to standard-of-care chemotherapeutics. In particular, in our models LS122 has anti-angiogenic and anti-invasion activities, it limited dissemination of cancer cells to distant sites *in vivo*, and LS122 has also blocked osteoblastic and osteolytic activation and function. We therefore propose in this modified SOW that, like the first application, we will study the role of a understudied kinase, RIP2K, in prostate cancer progression and to further evaluated LS122 has a possible drug for clinical development.

**NEW STATEMENT OF WORK AND TIMELINE FOR:
W81XWH-12-1-0011
NEW TITLE: TARGETING OF RIP2K TO INHIBIT CRPCa PROGRESSION
This new SOW was approved by Joshua McKean (appendix letter)**

Task 1 Establish Cell 3D co-culture models for sensitive and taxane resistant prostate cancer cells along with tumor associated fibroblasts.

Task 2 Test LS122 as a NF- κ B inhibitor via targeting RIP2K using 2D and 3D cell culture models.

- A. Demonstrate using 2D culture that LS122 is a potent inhibitor of NF- κ B and targets RIP2K. We propose to knockdown expression of RIP2K and MEKK3 to determine if they are the primary targets of LS122 responsible for regulating growth, motility and invasion of prostate tumor cells.
- B. Demonstrate that LS122 acts in 3D culture to block prostate cancer motility and invasion via inhibition of RIP2K. We propose to knockdown expression of RIP2K and MEKK3 to determine if they are the primary targets of LS122 responsible for regulating growth, motility and invasion of prostate tumor cells.
- C. Perform proliferation and apoptosis assays to determine if LS122 sensitizes PC3 and DU145 cells to taxanes in 2D and 3D culture

These proposed studies will be greatly aided using a high throughput real time imaging platform called the ZOOM incucyte. The P.I. directs the drug discovery and development core and has access to this imaging platform.

Task 3 Determine if RIP2K plays a role in osteoclasts/osteoblast function or differentiation . Preliminary results suggest LS122 alters the function and/or differentiation of bone localized cells critical in prostate cancer bone metastasis establishment and growth. These studies will be repeated and extended to determine effective doses of LS122 that work and the pathways it works on to target cells playing a key role in growth of PCa in the bone.

Task 3 Mouse studies to test LS122 in vivo (NOT FUNDED BY DOD) PARTIALLY COMPLETED AND WILL NOT BE COMPLETED USING DOD FUNDS

Task 4 Write up manuscript for submission. IN PROGRESS

KEY RESEARCH ACCOMPLISHMENTS

Patent for LS122 has been executed and we are now looking for licensing possibilities to move this drug closer to clinical trials for men with CRPCa.

RIP2K was identified as a main target of LS122 and a new SOW was prepared along with a six month extension to further study this.

LS122 worked to prevent metastasis in an animal model.

REPORTABLE OUTCOMES

A patent for LS122 was executed in the U.S. and Europe and we are searching for companies to license this technology.

CONCLUSION

We developed a 3D cell co-culture system. We screened a 422 member kinase panel and found LS122 inhibited >90% of RIP2 kinase activity, but had no significant activity on other kinases tested. RIP2K is a TNF- α responsive, upstream regulator of NF- κ B, a “druggable target”, and novel in prostate cancer. We verified that LS122 inhibits RIP2K phosphorylation at micromolar concentrations. In order to characterize the anti-cancer efficacy of LS122, we have conducted viability assays in PC-3, RM-1, LNCaP and its derivative C4-2, DU145 and other prostate cancer lines, as well as a set of non-tumor prostate cells including RWPE-1, WPMY-1, and 267B1. Additional non-prostate cells were also tested. In general, LS122 had minimal effect on viability of non-cancer cells, but demonstrated a dose-dependent effect on all prostate cancer lines and various other cancer types. LS122 was also a potent inhibitor of cancer cell motility and invasion in vitro in 2D and 3D models. In TNF- α stimulated PC-3 cells, LS122 significantly blocked a number of inflammatory, progression-related cytokines/chemokines. In vivo, LS122 had a significant, dose-dependent effect on tumor doubling, final tumor volume and tumor angiogenesis in PC-3 human xenografts and in the highly aggressive RM-1 syngeneic model. LS122 blocked the dissemination to the liver of cancer cells injected into the spleen by >90%; nearly double that of another experimental NF- κ B inhibitor of an entirely different drug class. LS122 inhibits the proliferation, ruffling, fusion and osteolytic activity of osteoclasts and inhibited the proliferation, alkaline phosphatase secretion and mineralization of osteoblasts.

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SUPPORTING DATA

DESCRIPTION OF FIGURES IN SUPPORT OF THE NEW SOW

FIGURE 1 DU145 tumor cells were grown on top of matrigel. After spheroids formed, HGF, the growth factor for the invasion and metastasis stimulating receptor Met, was added and cells were incubated for an additional 24 hours. HGF along with EGF (not shown) stimulate the formation of invasive cells into the matrigel.

FIGURE 2 DU145 tumor cells were grown on top of matrigel containing or not containing WPMY prostate fibroblasts. Without fibroblasts the tumor cells grow into noninvasive spheroids while in the presence of the fibroblasts, invasive structures form.

FIGURE 3 STRUCTURE OF LS122, a modified theophene belonging to the quinazoline class of drugs.

FIGURE 4 10 micromolar LS122 inhibits phosphorylation of the p65 subunit in the presence of TNF- α (left panel). In the right panel, LS122 and TNF alpha treated mice demonstrate lower levels of an NF-kB expression cassette (C) compared to control mice (A) or mice treated with TNF- α .

FIGURE 5 These studies were done in collaboration with the Agarwal laboratory, internationally recognized for studies on NF-kB. The cancer cell line SW480 was serum starved for 24 h, pretreated with 242-A, 242-NM and 242-bag (3 and 10 μ M) for 2 h and then exposed to TNF- α (10 ng/ml) for 10 min. Each of these drugs are different synthesized lots. Thereafter, nuclear and cytoplasmic fractions were prepared, and analyzed for NF-kB signaling molecules (p50, p65, I κ B α) and by Western blotting and NF-kB activation by EMSA. Western blot analyses showed that in SW480 cells, TNF- α exposure induced the nuclear localization of p50 with a corresponding slight decrease in the cytoplasmic p50 level (Figure 5). 242-A, 242-NM and 242-bag (3 and 10 μ M each) treatment strongly inhibited the p50 nuclear localization in SW480 cell with an increase in cytoplasmic p50 level (Figure 5). TNF- α exposure also increased the nuclear p65 level in SW480 cells along with a clear decrease in cytoplasmic p65 level (Figure 5). Even though an increase in cytoplasmic p65 was observed with 242-A (3 and 10 μ M) a corresponding decrease in nuclear p65 level was not clearly evident (Figure 5). Both, 242-NM and 242-bag decreased the nuclear p65 expression but an increase in cytoplasmic p65 was observed only with 242-bag (Figure 5). I κ B α is known to inhibit NF-kB activation, and our results showed that TNF α exposure resulted in a decrease in I κ B α expression in SW480 cells (Figure 5). In general, all the three compounds increased the I κ B α expression even though with different potency (Figure 5). Membranes were also stripped and re-probed for PARP as nuclear loading control and for tubulin as cytoplasmic loading control (Figure 5). Additionally experiments are being performed with DU145 and PC3 prostate cancer cells.

FIGURE 6 Western blot analyses showed the effects of 242-A, 242-NM and 242-bag (these are different lots of the same drug) on the expression and localization of NF-kB signaling molecules (p50, p65, and I κ B α); therefore to clearly understand the effect of these three drugs on NF-kB activation we performed EMSA. In both SW480 and LoVo cells (not shown), TNF α exposure activated the NF-kB, measured in terms of its DNA binding (Figure 6). The treatment with 242-A, 242-NM and 242-bag (3 and 10 μ M each) strongly inhibited the TNF α -induced NF-kB activation in SW480 cells at both the doses (Figure 3, Left Panel). The specificity of EMSA bands as well as constituents of NF-kB were determined by super-shift and competition assays, respectively. Competition assay confirmed the position and specificity of NF-kB

bands ((Figure 6, Middle Panel). In super-shift assay, nuclear extracts (TNF α exposed samples from SW480 and LoVo cells) were first incubated with either anti-p50 or anti-p65 antibody followed by EMSA. This assay showed a strong super shift to a higher molecular weight band in case of both anti-p50 and anti-p65 antibodies, suggesting that the observed NF- κ B band consisted of these two subunits.

FIGURE 7 Quinazoline drugs are often kinase inhibitors. For example, erlotinib and gefitinib are 2 well-studied drugs of this class that have shown efficacy in some solid tumors, but have also been plagued with off-target effects against a wide variety of receptor tyrosine kinases and dose-limiting cardiotoxicities. LS122, along with all other hits from our initial reporter cell screen including all thiophene derivatives were analyzed [at DiscoverX, previously KINOMEScan/Ambit Biosciences, San Diego, CA] for inhibition against a panel of 422 kinases (Figure 7 left panel). LS122 significantly inhibited only 4 kinases; most potently, RIPK2. Two of the other kinases, ZAK and TNK1, were in the same family, while STK36 (fused), the kinase responsible for nuclear translocation of Gli-1 downstream of sonic hedgehog activation, was the fourth kinase significantly inhibited by LS122 but further assays we performed indicated this kinase was not inhibited by LS122. We validated that in PC-3 cells; LS122 inhibited phospho-RIPK2 independent of TNF- α stimulation (Figure 7 bottom right panel), but had no effect on RIP2 total protein levels.

FIGURE 8 A model demonstrating the different pathways leading to activation of NF- κ B. We propose that LS122 inhibits RIP2K which in turn acts on MEKK3 to initiate signaling to NF- κ B.

FIGURE 9 PC-3 cells were treated \pm 10 μ M LS122. Total mRNA was isolated for hybridization to Signal Transduction Pathway Finder oligo arrays (SA Biosciences) as described by the manufacturer. The resulting images were quantitated using ImageQuant and all results presented below represent decreases greater than 50% of control. LS122 was selected as an NF- κ B and AP-1 dual inhibitor, and this experiment confirmed that many members of the NF κ B and AP-1 pathways were affected by LS122 treatment.

FIGURE 10 The Essen Bioscience ZOOM Incucyte real time imaging platform was used to measure motility of PC3 cells. The top panel represents PC3 cells in the presence or absence of the growth factor HGF and LS122 at 20 micromolar. At this concentration, LS122 blocks PC3 motility plus or minus HGF. The bottom panel shows comparable data for 10 micromolar LS122. LS122 also blocks invasion of PC3 cells through matrigel using the Incucyte system (not shown).

FIGURE 11 The tumor microenvironment is altered during cancer progression and a drug with effects on this compartment in addition to the tumor cell could be particularly attractive. RIPK2's reported regulation of RANKL production from osteoblasts is highly significant to the present proposal. In addition to expression in PC-3 cells, RIPK2 is expressed in osteoblasts and controls a NOD1/2-modulated signaling cascade that results in the release of inflammatory cytokines and RANKL that stimulate the bone microenvironment to elicit the —vicious cycle of bone destruction. As illustrated in Figure 11, our preliminary in vitro studies show that LS122 blocks key steps in osteoblast and osteoclast activation. For example, osteoblast proliferation, alkaline phosphatase secretion and mineral deposition are inhibited. Also, in osteoclasts, LS122 inhibited RANKL-stimulated proliferation, as well as ruffling and fusion, expression of key osteogenic factors such as OPN, cathepsin K, and TRAP, and blocked destruction of mineralized bone. These studies will be confirmed in the new SOW.

FIGURE 12-13 In vivo anti-cancer assays: As a regulator of NF- κ B and AP-1 signaling, LS122 may play an important role in many facets of prostate cancer progression and metastasis. We have found LS122 to

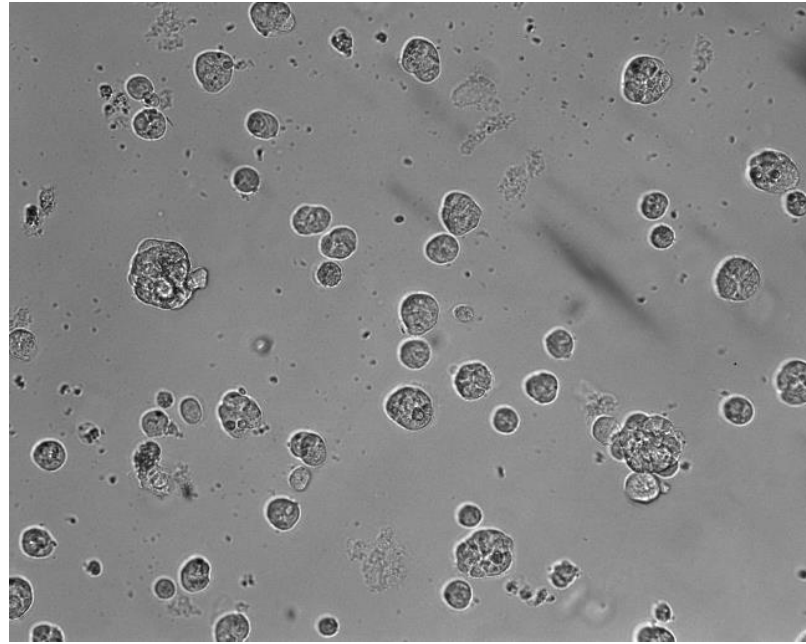
be efficacious in a variety of standard anti-cancer assays. For example, as illustrated in Figure 12 and 13, LS122 significantly limited the tumor volume in 2 subcutaneous xenografts models; PC-3 and syngeneic RM-1. LS122 significantly decreased expression of VEGF and other angiogenic factors in cultured cells (data not shown) and potently reduced the number of new vessels in xenograft PC-3 and syngeneic RM-1 prostate tumors (12B).

FIGURE 14 No significant organ toxicity has been seen with daily delivery of LS122 at 50 mg/kg oral or IP for as long as 90 days, nor did mice lose any body weight or show signs of systemic toxicity, making LS122 a potentially efficacious and low toxicity drug.

FIGURE 15 In the organ to organ metastasis model, tumor cells were directly injected into the spleen (n=5 per group) and allowed to migrate to the liver as an aggressive experimental metastasis model. Spleens from all mice were populated with numerous tumor nodules. Livers from untreated mice all had significant numbers of large tumor nodules; however, only one small nodule was found in one liver of LS122 treated mice.

FIGURE 1

CONTROL



HGF

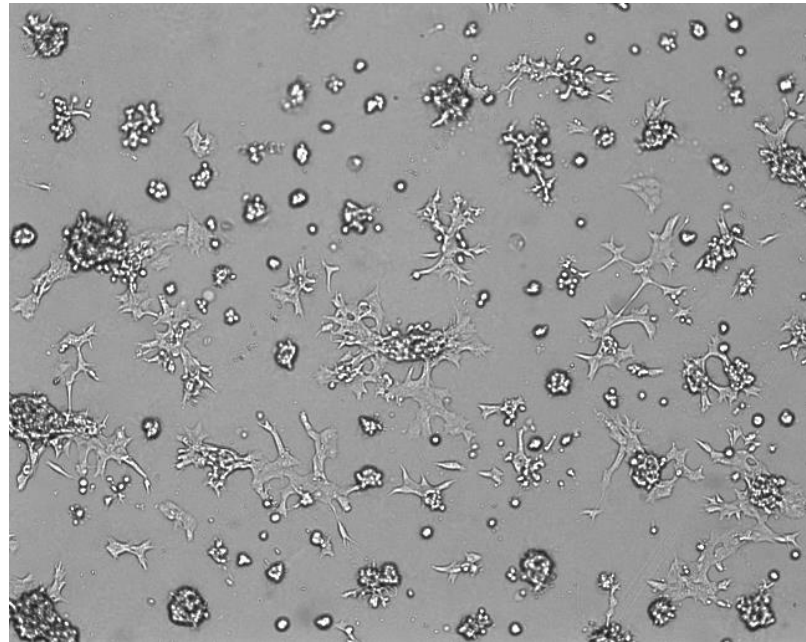
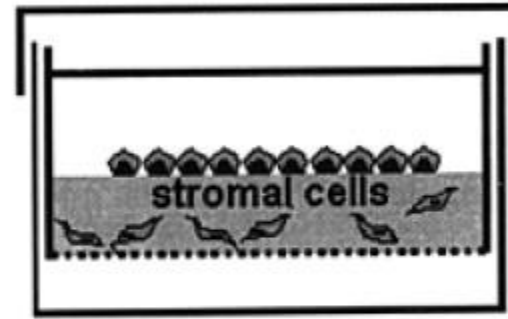
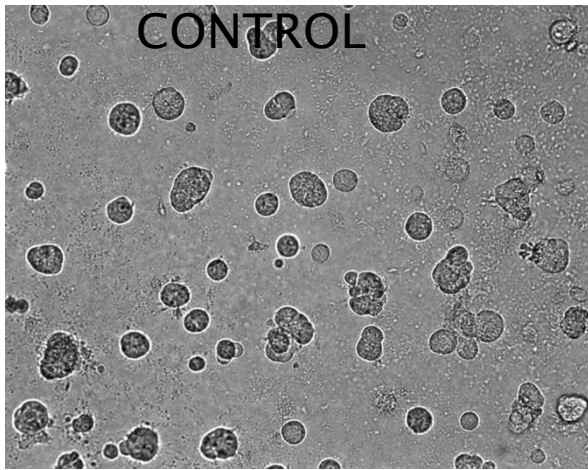


FIGURE 2

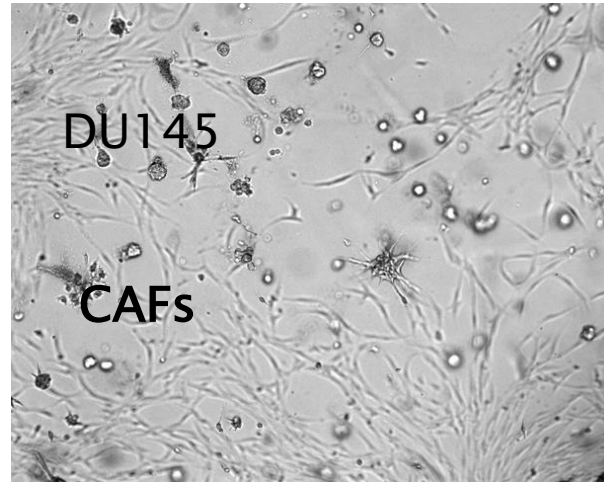
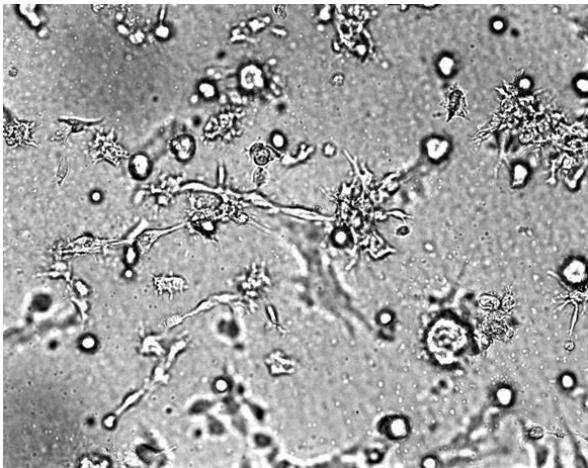
DU145



**Co-culture with
Stromal Cells in BME**

DU145

CAFs



OPTICAL FOCUS ON DU145 CELLS

FOCUS ON FIBROBLASTS

LS122: novel thiophene derivative dual NF κ B/AP-1 inhibitor

3-(4-acetyl-phenyl)-2-(5-morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3Hquinazolin-4-one

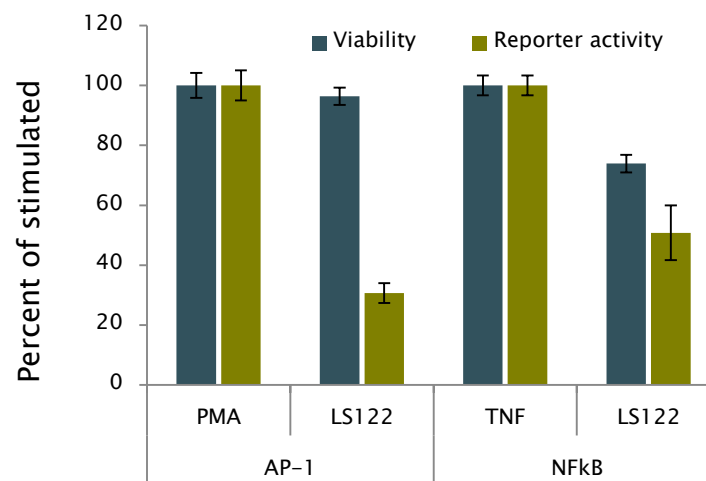
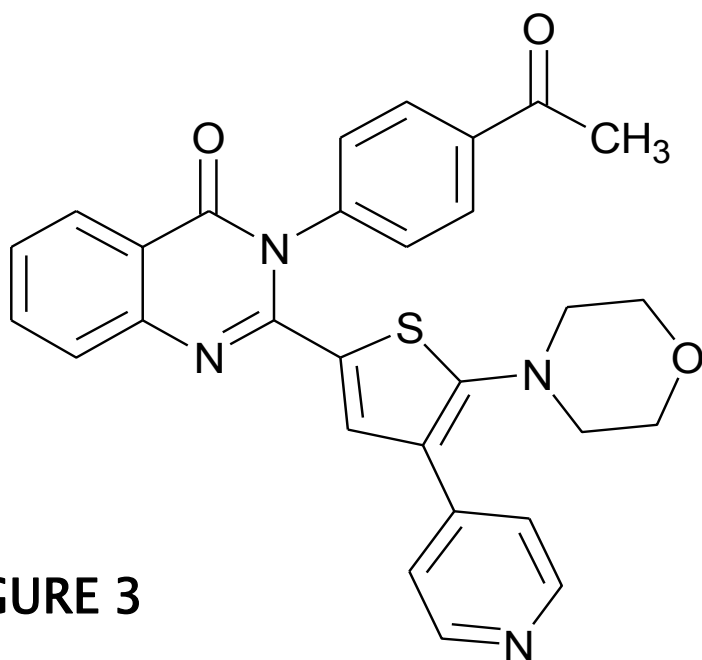


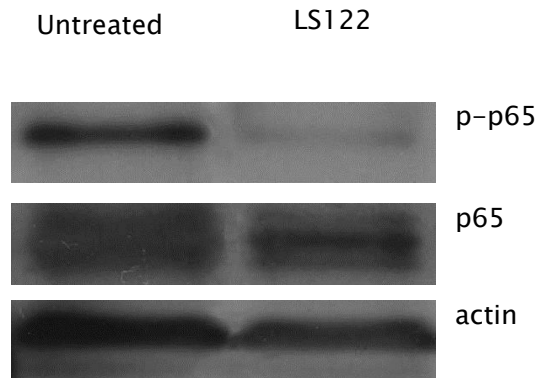
FIGURE 3

Initial screening at 10 μ M

LS122 decreased *in vitro* and *in vivo* targets consistent with an NFκB inhibitor

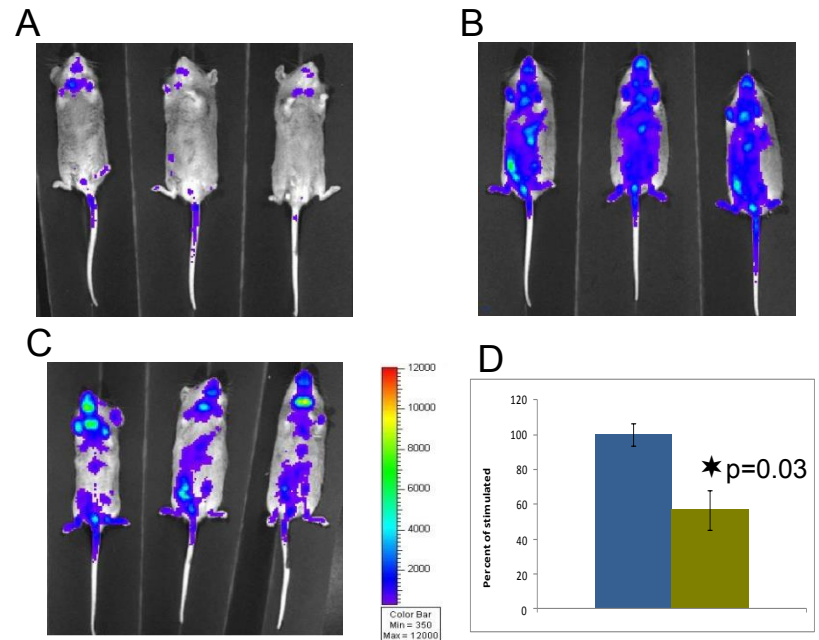
FIGURE 4

In vitro



Phospho-p65 NFκB subunit expression decreased

In vivo



NFκB reporter mice had 43% less expression

FIGURE 5

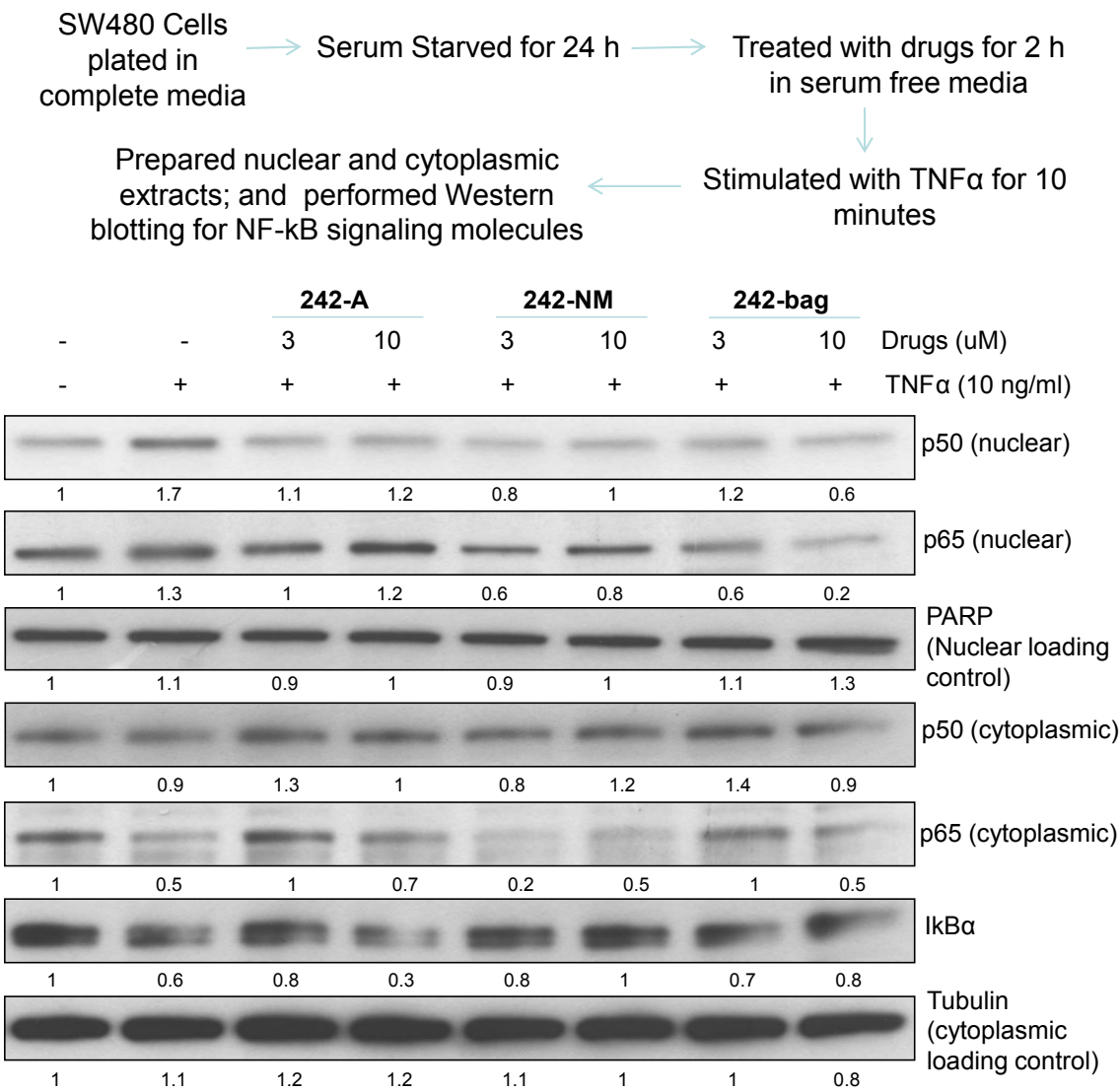


Figure 1

FIGURE 6

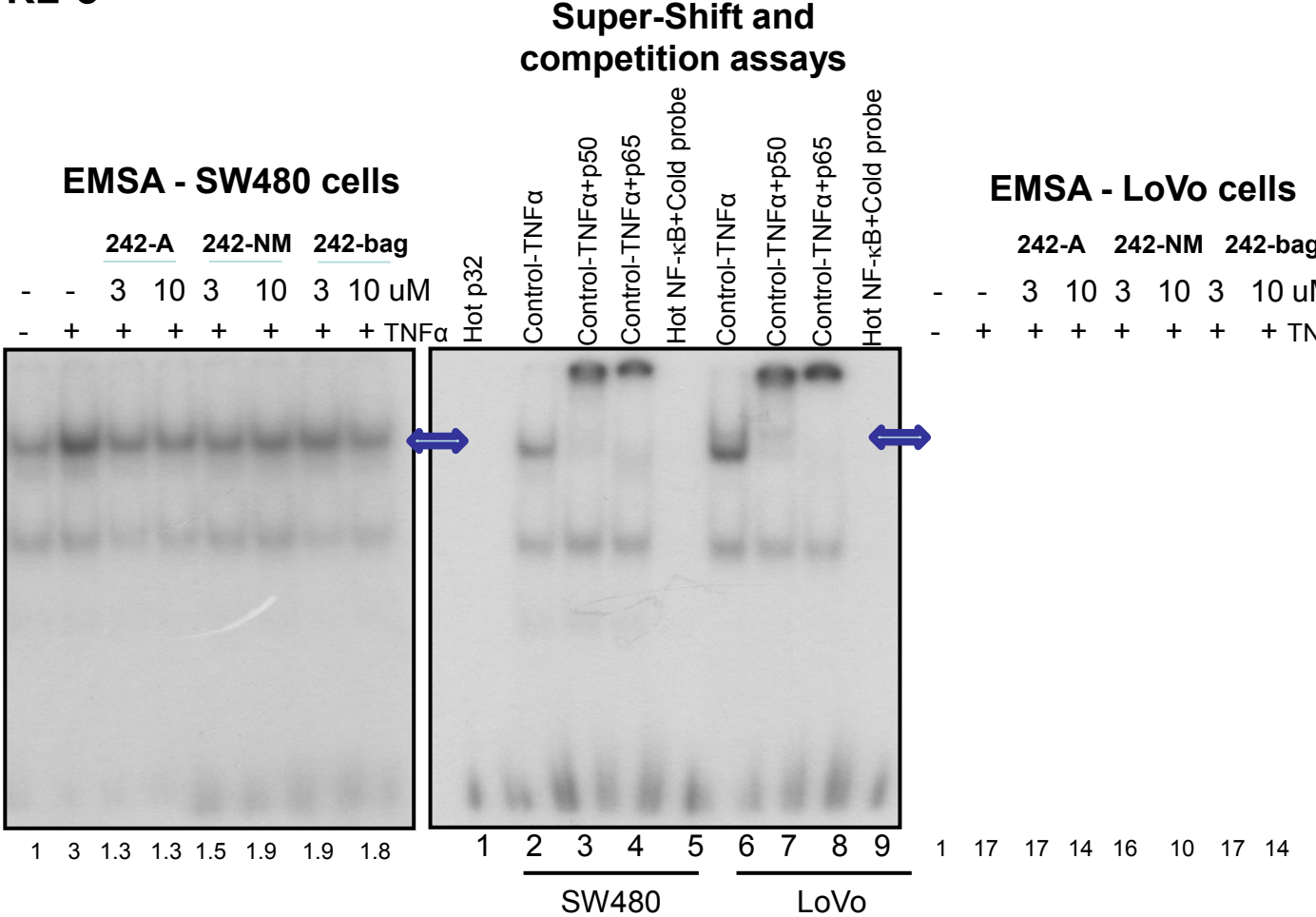
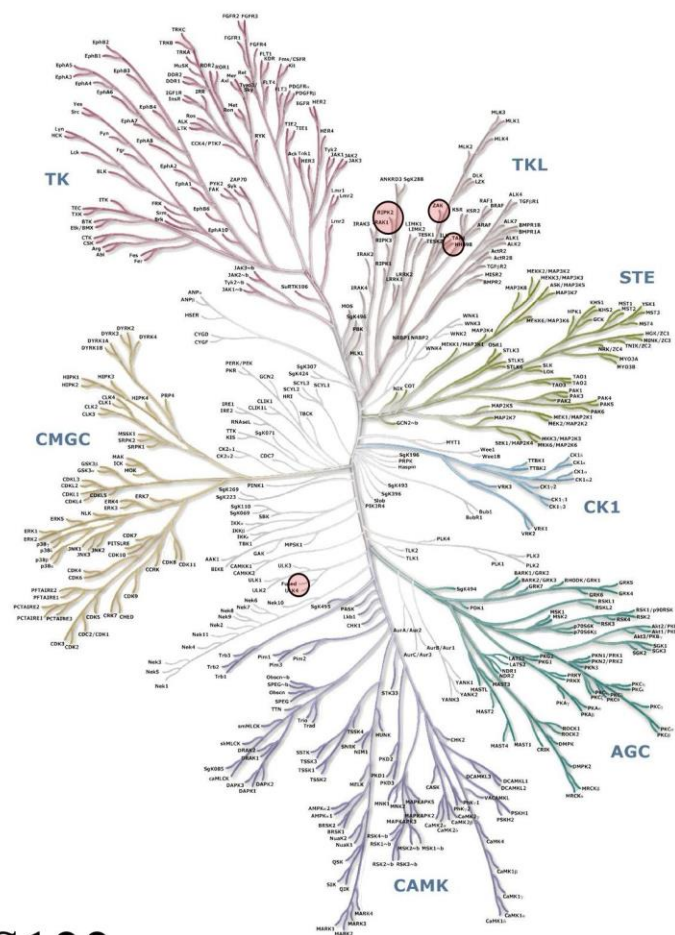


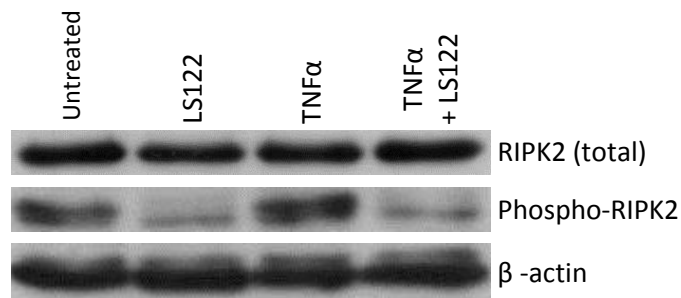
Figure 3

LS122 is most selective for RIPK2, a kinase relevant to cancer progression

FIGURE 7



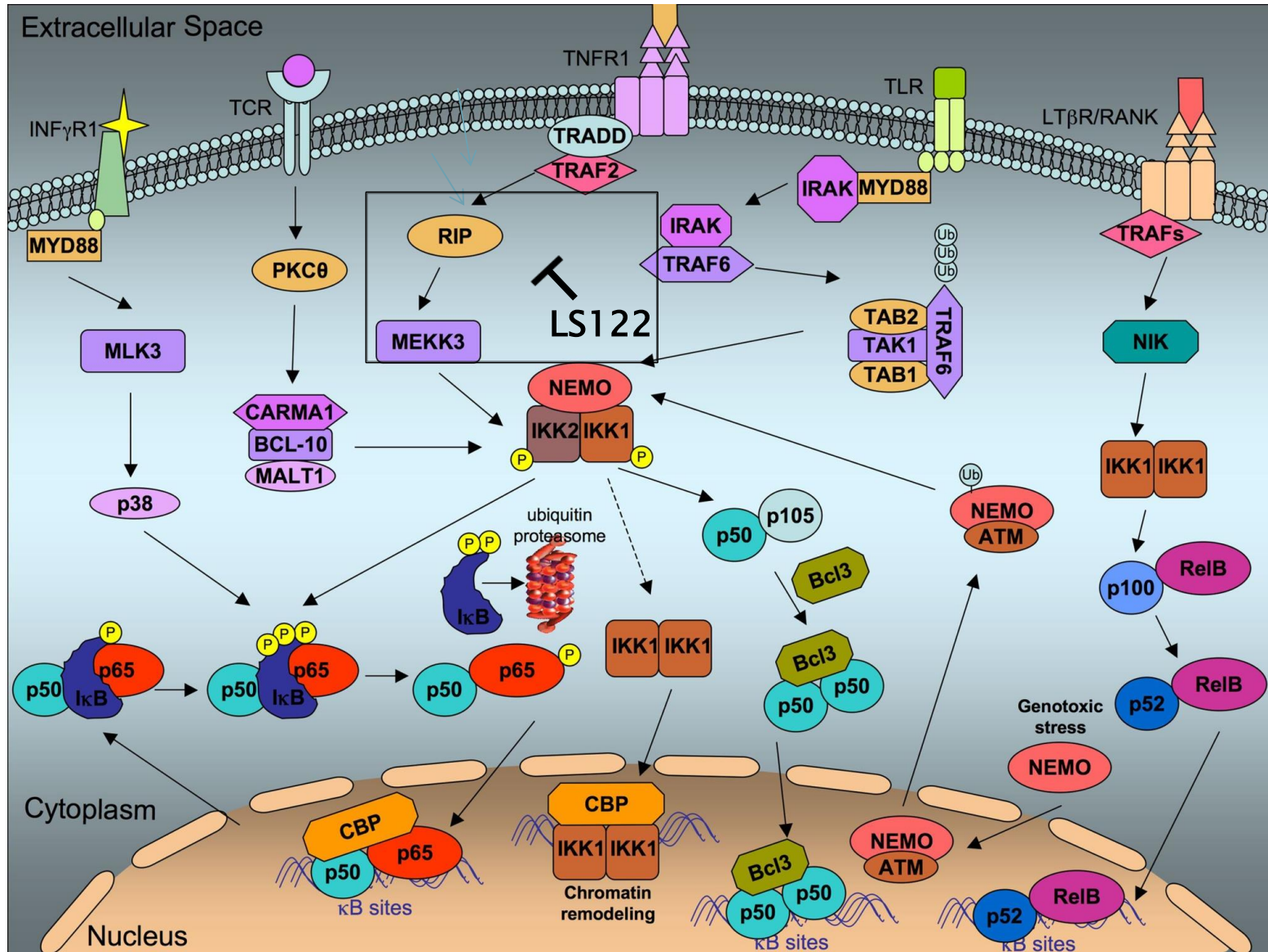
Ambit Gene Symbol	Percent Inhibition
RIPK2	91
STK36	83
ZAK	73
TNNI3K	70



All other thiophene derivatives and all hits from the NFκB and AP-1 reporter screens were evaluated for RIPK2 and 5 relevant kinases (relevant biologically to us at the time) and no other compound was a RIPK2 inhibitor of ANY magnitude.

LS122
442 Total kinases tested
78 Kinases not mapped

FIGURE 8 LS122 most likely targets RIP2K to inhibit NFKB activation



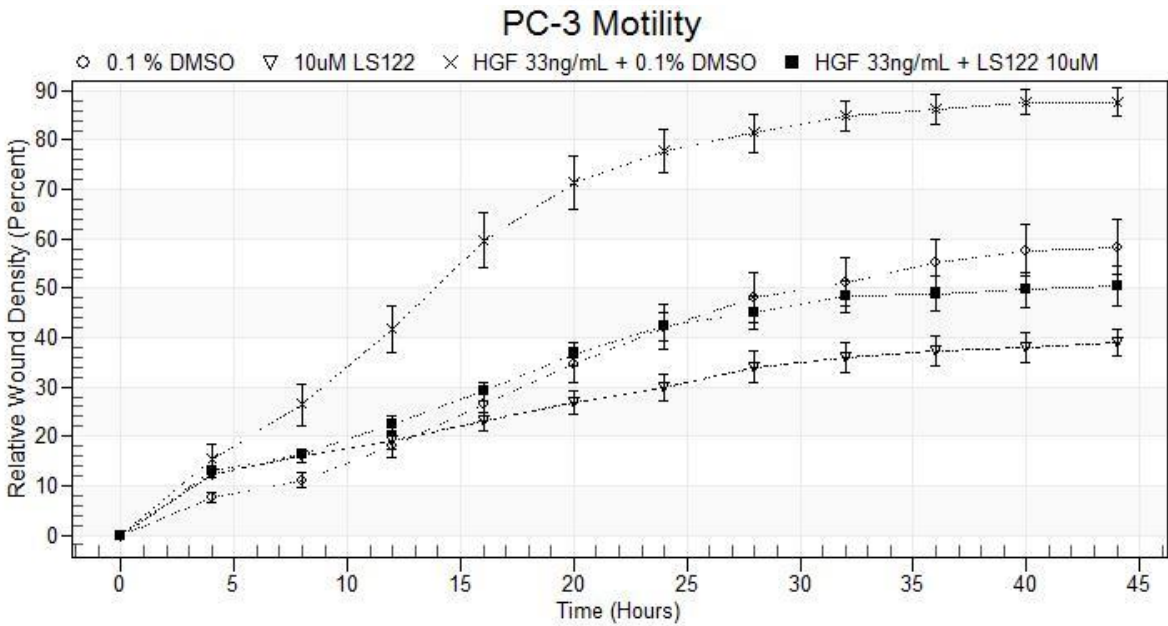
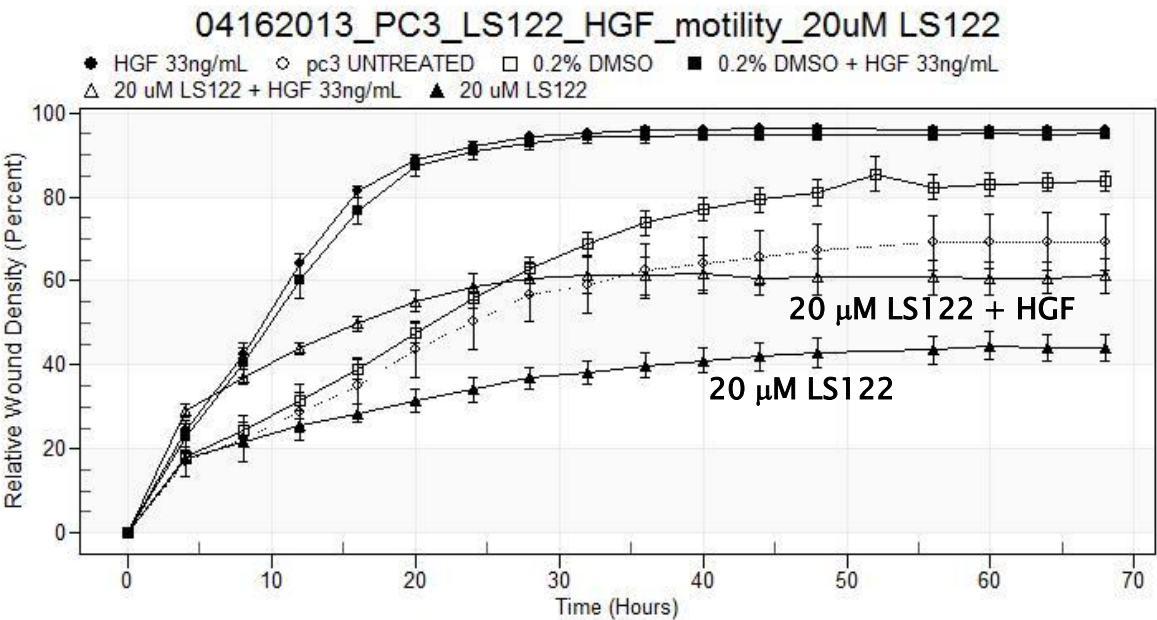
Key NFkB signal transduction pathway members are decreased by treatment with LS122

FIGURE 9

▣ NFkB pathway

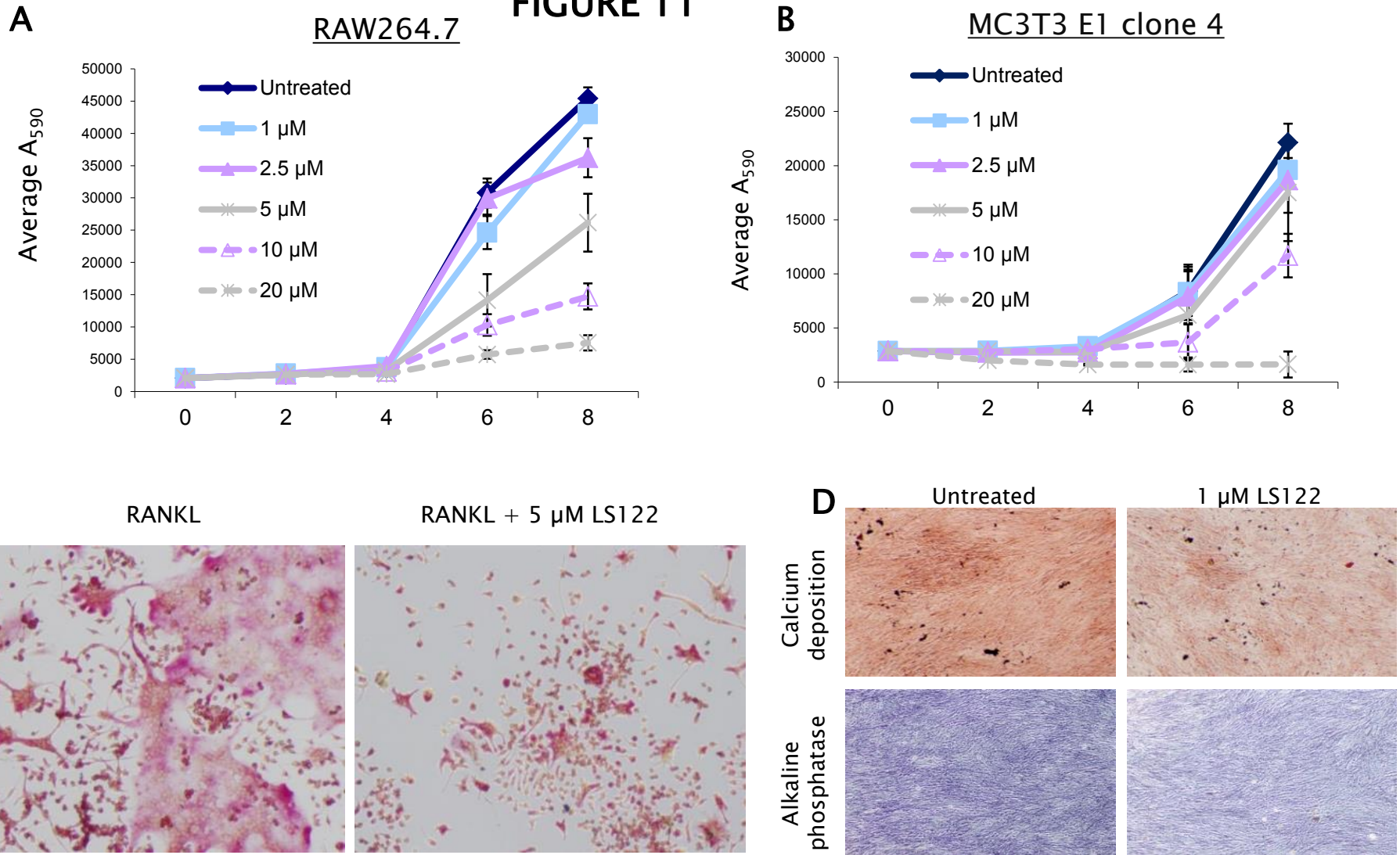
- ▣- **NAIP** Apoptosis /Anti-apoptosis
- ▣- **BIRC 2** Protein binding; Apoptosis/Anti-apoptosis; Positive regulation of I-kappaB kinase/NF-kappaB cascade
- ▣- **BIRC3** Protein binding; Apoptosis/Anti-apoptosis; Ubiquitination
- ▣- **CCL20** Amino acid phosphorylation; Cell adhesion; Anti-apoptosis; Humoral immune response; Inflammatory response
- ▣- **IL-8** Immune response; Intracellular signaling cascade; Negative regulation of cell proliferation
- ▣- **NFkB** Signal transduction; Regulation of transcription, Apoptosis/Anti-apoptosis; Transcription factor activities
- ▣- **IKBa** Cytoplasm; Apoptosis; Response to bacteria; Transcription factor binding; Sequestering of NFkB
- ▣- **TERT** Transferase activity; DNA binding; Telomerase-dependent telomere maintenance; RNA binding
- ▣- **Cox-2** Oxidoreductase activity; Peroxidase activity; Cell motility; Regulation of inflammatory response; Oxidoreductase

FIGURE 10



LS122 blocks key steps in both osteoblast and osteoclast activation

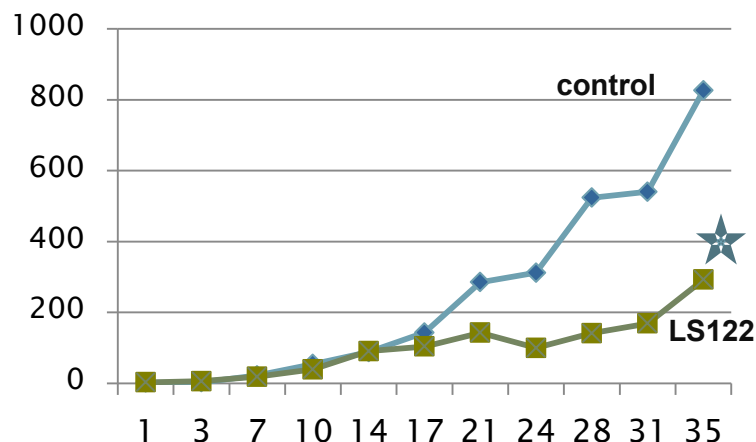
FIGURE 11



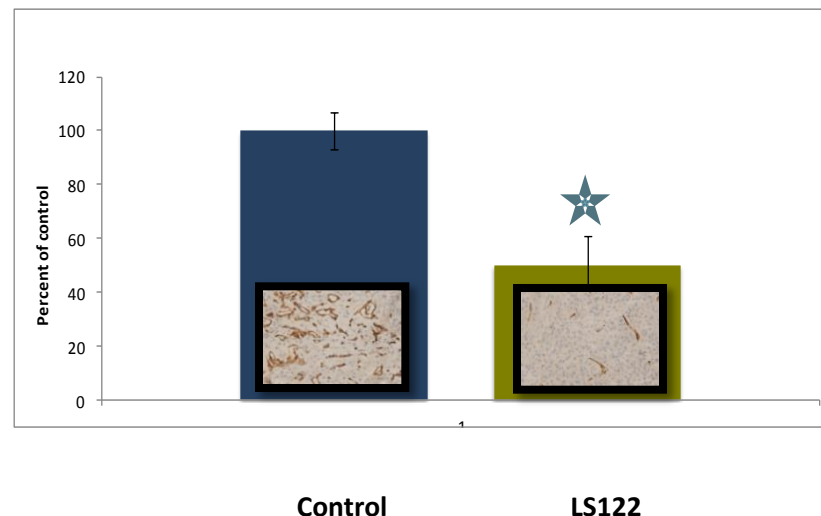
LS122 is a potent inhibitor of PC-3 human prostate cancer cell growth and angiogenesis *in vivo*

FIGURE 12

A.



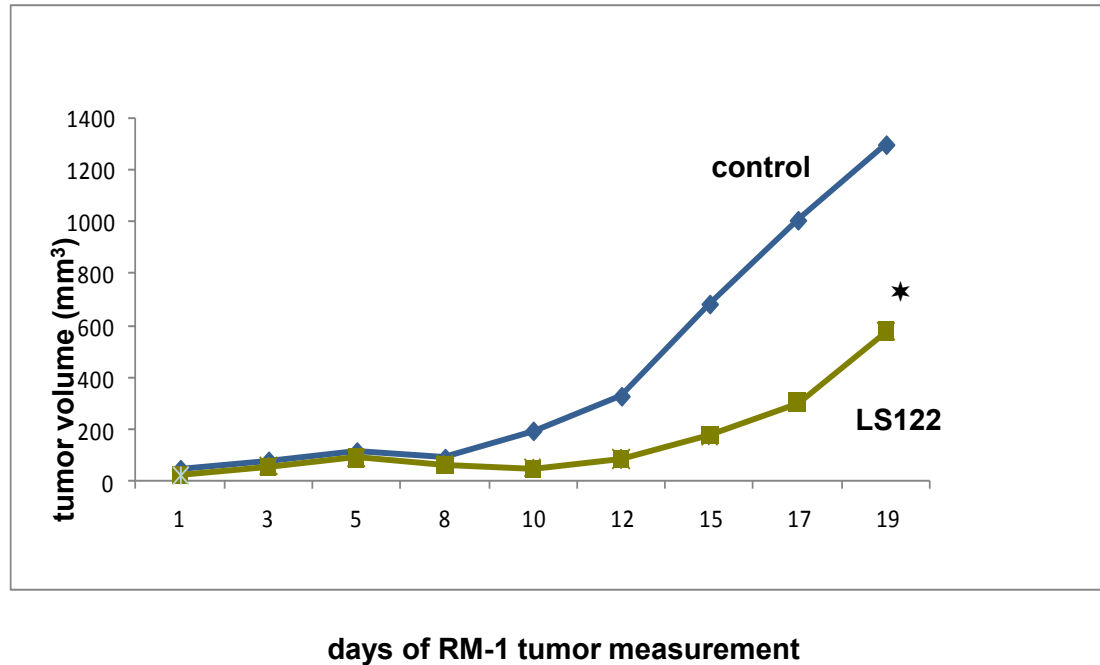
B.



A) PC-3 cells (1×10^6) were injected sc into SCID/bg mice. The mice were treated daily by oral gavage with 50mg/kg LS122 in 0.2% Noble agar or agar alone. Tumors were measured with calipers 3 times per week for 35 days. Tumor volumes were calculated using the standard formula $[\text{vol} = 0.52 \times L \times W^2]$, $\star p = 0.02$. **B)** Tumors harvested from animals in Panel A were paraffin embedded, sectioned, and stained for CD34, a marker for angiogenesis. Mean microvessel density was calculated from 5 random images of each tumor (insets show representative images). Results shown are the average \pm SEM, $\star p < 0.001$. Staining was quantified using ImageJ.

LS122 decreased tumor growth in aggressive RM-1 syngeneic tumor model

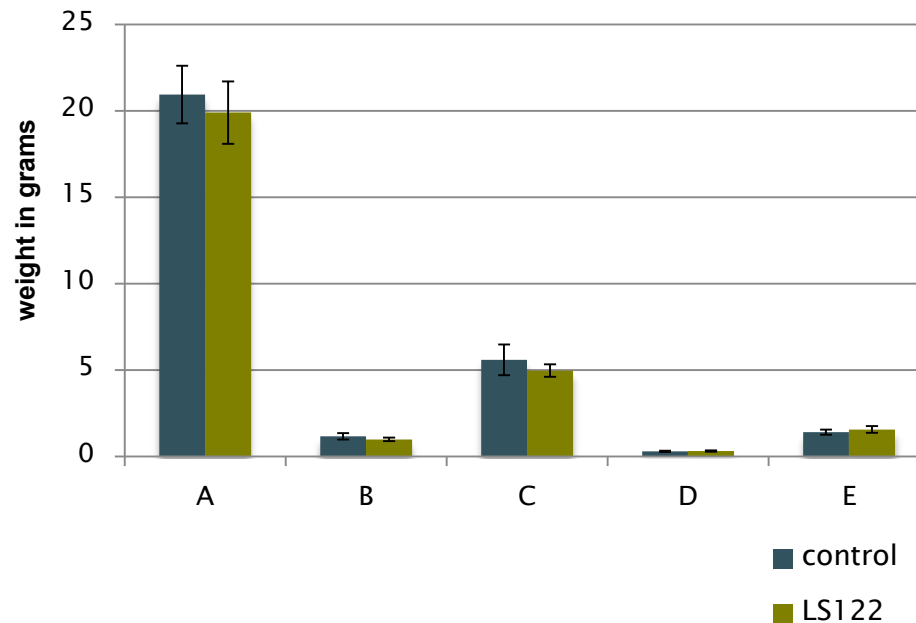
FIGURE 13



Male C57Bl/6 mice (8 weeks old) were injected in the right flank with 1×10^5 RM-1 mouse prostate cancer cells. Animals were treated daily with 0.2% Noble agar alone (control) or 50 mg/kg LS122 in 0.2% Noble agar immediately after tumor cell inoculation. Mice were measured 3 times per week by caliper. The average tumor volume ($n=10$) is shown. LS122 dramatically restricted the tumor size compared to the control animals, $\star p = 0.02$.

LS122 had no effect on body or organ weight and no significant gross or microscopic toxicity

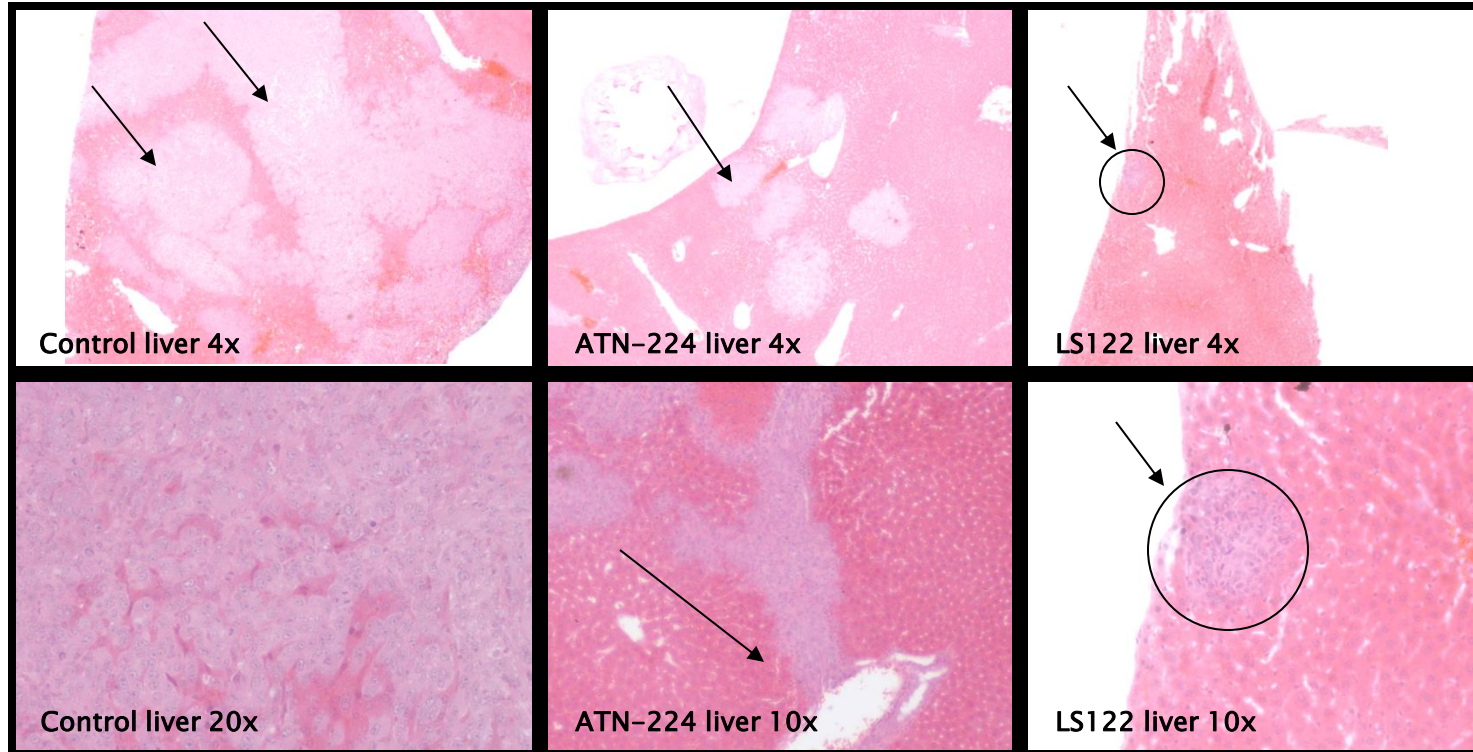
FIGURE 14



Male SCID/bg mice were given standard chow \pm LS122. Mice were sacrificed after 30 days, at which time their **A)** body weight, **B)** liver, **C)** liver to body ratio, **D)** kidney weight and **E)** kidney to body ratio were calculated. The averages from the mice in each group are shown. LS122 has no gross adverse effects on the mice.

LS122 blocks organ-to-organ dissemination and invasion

FIGURE 15





Health Sciences Center

SCHOOL OF MEDICINE IN SHREVEPORT

Department of Microbiology and Immunology

School of Medicine in Shreveport
School of Allied Health Professions
School of Graduate Studies
LSU Hospital in Shreveport
E.A. Conway Medical Center in Monroe

Joshua McKean
Grants Specialist
USAMRMC
Fort Detrick, MD 21702

April 22, 2013

Dear Joshua,

I hope this letter finds you doing well. With this letter and supporting documentation I am formally requesting a 7.5 month no cost extension (5-15-13 to 12-31-13) for contract W81XWH-12-1-0011. The supporting material includes a progress report for both the original proposal and the new modified proposal. The attachment also includes a new statement of work (SOW). I have also included an updated budget of remaining funds how they could best be distributed into support categories. Please note that although the progress report includes important proof of principle animal studies testing LS122, this was done using **LSU funds and approved by our IACUC**. The original allocated funds proposed to be used for animal will be rolled into supplies.

You will read in the attached that the original hypothesis that LS122 was a potent inhibitor of the Hedgehog signaling pathway proved to be incorrect, and this forced us to reformulate our hypothesis and approach. Also documented is the fact that the project was delayed due to circumstances beyond my control. Fortunately, it turns out that LS122 does target an unexpected member upstream in the NF- κ B pathway, and in associated documents, we provide compelling evidence that this compound could be developed to treat castrate resistant prostate cancer. Thus, our new SOW is similar in manner ways to the old SOW except that the therapeutic target under investigation has changed. Please let me know if you need any additional information. I am excited about our progress, and I hope we are allowed the opportunity to successful finish our work assignments over the next few months.

Sincerely,

James A. Cardelli, PhD

Professor of Microbiology and Immunology LSU Health Sciences Center
Director of Basic and Translational Research – Feist-Weiller Cancer Center
Director of the Innovative North Louisiana Experimental Therapeutics Program



LSUHealthShreveport

HEALTH SCIENCES CENTER

May 6, 2013

Mr. Joshua McKean
Grants Specialist
USAMRMC
Fort Detrick, MD 21702

RE: Grant No. W81XWH-12-1-0011

Dear Joshua,

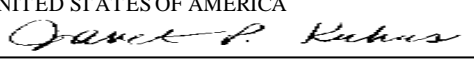
Please see the attached documentation regarding Grant No. 81XWH-12-1-0011. The Institution supports the request for a no-cost extension and the submission of a new, modified statement of work for this project.

Please contact the Office for Sponsored Programs (318-675-7884/ grants@lsushc.edu) should you have any questions or need additional information.

Regards,

Hugh E. Mighty, MD, MBA, FACOG
Vice Chancellor for Clinical Affairs

CC: Enclosures

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT				1. CONTRACT ID CODE S		PAGE OF PAGES 1 11	
2. AMENDMENT/MODIFICATION NO. P00002		3. EFFECTIVE DATE 14-May-2013		4. REQUISITION/PURCHASE REQ. NO. W91ZSQ1295N652		5. PROJECT NO.(If applicable)	
6. ISSUED BY USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014		CODE W81XWH		7. ADMINISTERED BY (If other than item 6) US ARMY MEDICAL RESEARCH ACQUISITION ACT ATTN: JOSHUA MCKEAN 301-619-4046 FORT DETRICK MD 21702		CODE W81XWH	
8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code) LOUISIANA STATE UNIVERSITY SYSTEM 1501 KINGS HWY SHREVEPORT LA 71103-4228				9A. AMENDMENT OF SOLICITATION NO.			
				9B. DATED (SEE ITEM 11)			
				X 10A. MOD. OF CONTRACT/ORDER NO. W81XWH-12-1-0011			
				X 10B. DATED (SEE ITEM 13) 15-May-2012			
CODE 0V3R3		FACILITY CODE					
11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS							
<input type="checkbox"/> The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer <input type="checkbox"/> is extended, <input type="checkbox"/> is not extended. Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.							
12. ACCOUNTING AND APPROPRIATION DATA (If required)							
13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.							
A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.							
B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).							
C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:							
X D. OTHER (Specify type of modification and authority) IAW USAMRAA General Terms and Conditions							
E. IMPORTANT: Contractor <input checked="" type="checkbox"/> is not, <input type="checkbox"/> is required to sign this document and return _____ copies to the issuing office.							
14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.) Modification Control Number: jmclean133713 1. The purpose of this modification is to extend the period of performance, without additional funding, from 15 May 2012 - 14 May 2013 to 15 May 2012 - 31 December 2013. An annual progress report has been submitted on 22 April 2013. The final technical report will now be due on 31 December 2013. 2. Additionally, the revised budget and revised Statement of Work, both dated 22 April 2013, will be incorporated herein by reference. 3. All other terms and conditions of the award remain unchanged.							
Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.							
15A. NAME AND TITLE OF SIGNER (Type or print)				16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) JANET P KUHNIS / CONTRACTING OFFICER TEL: 301-619-2827 EMAIL: janet.p.kuhns.civ@mail.mil			
15B. CONTRACTOR/OFFEROR (Signature of person authorized to sign)		15C. DATE SIGNED		16B. UNITED STATES OF AMERICA BY  (Signature of Contracting Officer)		16C. DATE SIGNED 10-May-2013	

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

CLIN 0001

The CLIN extended description has changed from Period of Performance: 15 May 2012-14 May 2013FY11 Prostate Cancer Research Program-Exploration/Hypothesis Development Award to Period of Performance: 15 May 2012-31 December 2013FY11 Prostate Cancer Research Program-Exploration/Hypothesis Development Award.

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 15-MAY-2012 TO 14-MAY-2013	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 15-MAY-2012 TO 31-DEC-2013	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

TERMS AND CONDITIONS
U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND (USAMRMC)
U.S. ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY (USAMRAA)
Effective October 1, 2011

TERMS AND CONDITIONS INCORPORATED BY REFERENCE (APRIL 2012)

The recipient shall comply with the terms and conditions below that are applicable to its type of organization:

a. **For Educational and Non-Profit Organizations:** This award incorporates by reference, with the same force and effect as if they were included in full text, the Research Terms and Conditions dated June 2011 (<http://www.nsf.gov/awards/managing/rtc.jsp>) and the USAMRAA Agency Specific Requirements dated October 1, 2011 (<https://www.usamraa.army.mil>).

b. **For Commercial (For-Profit) Organizations:** This award incorporates by reference, with the same force and effect as if they were included in full text, the USAMRAA General Terms and Conditions for Assistance Awards with For-Profit Organizations dated April 1, 2012 (<https://www.usamraa.army.mil>).

Any apparent inconsistency between Federal statutes and regulations and the terms and conditions contained in this award shall be referred to the USAMRAA Contract/Grants Specialist for guidance.

AWARD SPECIFIC TERMS AND CONDITIONS

This award is made under the authority of 31 U.S.C. 6304 and 10 U.S.C. 2358. The recipient's revised Statement of Work (SOW), dated 22 April 2013, and the revised budget, dated 22 April 2013 for the application submitted in response to the Fiscal Year 2011 Department of Defense (DOD) Prostate Cancer Research Program Exploration/Hypothesis Development Award Announcement (Funding Opportunity Number: W81XHW-11-PCRP-EHDA), which closed 8 June 2011, are incorporated herein by reference. The Catalog of Federal Domestic Assistance Number relative to this award is CFDA 12.420.

ACCEPTANCE OF AWARD

The recipient is not required to countersign this award. In case of disagreement, the recipient shall notify the USAMRAA Grants Officer and not assess the award any costs until such disagreement(s) is resolved.

ADMINISTRATIVE AND COST PRINCIPLES

The following Administrative and Cost Principles, as applicable, effective the earlier of (i) the start date of this award or (ii) the date on which the recipient incurs costs to be assessed against the award, are incorporated as part of this award by reference:

- a. CFR, Title 2, Part 220, "Cost Principles for Educational Institutions (OMB Circular A-21)."
- b. CFR, Title 2, Part 225, "Cost Principles for State, Local, and Indian Tribal Governments (OMB Circular A-87)."
- c. OMB Circular A-102, "Grants and Cooperative Agreements with State and Local Governments."
- d. CFR, Title 2, Part 215, "Uniform Administrative Requirements for Grants and Agreements with Institutions of Higher Education, Hospitals, and Other Non-profit Organizations (OMB Circular A-110)."
- e. CFR, Title 2, Part 230, "Cost Principles for Non-profit Organizations (OMB Circular A-122)." [For those nonprofit organizations specifically excluded from the provisions of OMB Circular A-122, Subpart 31.2 of the Federal Acquisition Regulations (FAR 48 CFR Subpart 31.2) shall apply].
- f. OMB Circular A-133, "Audits of States, Local Governments, and Non-Profit Organizations."
- g. Federal Acquisition Regulation, Part 31.2, for Commercial Organizations and those nonprofit organizations specifically excluded from the provisions of OMB Circular A-122.
- h. Department of Defense Grant and Agreement Regulations 3210.6-R.

These publications may be obtained from:

Office of Management and Budget
EOP Publications Office
New Executive Office Building
725 17th Street, NW, Room 2200
Washington, DC 20503
Telephone: (202) 395-7332
Website: <http://www.whitehouse.gov/omb/>

RECIPIENT RESPONSIBILITY

In addition to the responsibilities of the recipient as defined in the award or incorporated by reference herein:

- a. The recipient will bear primary responsibility for the conduct of the research and will exercise sound judgment within the limits of the award's terms and conditions.
- b. The Principal Investigator(s) (PI) specified in the award document will be continuously responsible for the conduct of the research project and will be closely involved with the research effort. The PI, operating within the policies of the recipient, is in the best position to determine the means by which the research may be conducted most effectively.
- c. The recipient shall request the USAMRAA Grants Officer's prior approval to change the PI or any key personnel, for the PI or any key personnel to be absent from the project during any continuous period of 3 months or more, or for the PI or any key personnel to reduce time devoted to the project by 25 percent or more from the level that was approved at the time of award.

KEY PERSONNEL

In addition to the PI, the following individual(s) is (are) identified as key personnel:

Dr. Jennifer L. Carroll, Research Assistant

AWARD MODIFICATION

The only method by which this award may be modified is by a formal, written modification signed by the USAMRAA Grants Officer. No other communications, whether oral or in writing, are valid to change the terms and conditions of this award. See the USAMRAA Agency Specific Requirements for changes requiring USAMRAA Grants Officer's prior approval.

MAXIMUM OBLIGATION

The maximum obligation of the Government for support of this award will not exceed the amount specified in the award, as modified. Awards will not be modified to provide additional funds for such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits, and other costs.

SUPPORTING INFORMATION

Information such as subawards, consultant agreements, vendor quotes, and personnel work agreements may be required in order to support proposed costs or to determine the employment status of personnel. The Government's receipt of this information does not constitute approval or acceptance of any term or condition included therein.

FINANCIAL INSTABILITY, INSOLVENCY, BANKRUPTCY OR RECEIVERSHIP

a. The recipient shall immediately notify the USAMRAA Grants Officer of the occurrence of the following events: (1) the recipient's financial instability that would negatively impact performance of this award; (2) the recipient's or recipient's parent's filing of a voluntary case seeking liquidation or reorganization under the Bankruptcy Act; (3) the recipient's consent to the institution of an involuntary case under the Bankruptcy Act against the organization or organization's parent; (4) the filing of any similar proceeding for or against the recipient or recipient's parent, or its consent to, the dissolution, winding-up or readjustment of the recipient's debts, appointment of a receiver, conservator, trustee, or other officer with similar powers over the organization, under any other applicable state or federal law; or (5) the recipient's insolvency due to its inability to pay its debts generally as they become due.

b. Such notification shall be in writing and shall: (1) specifically set out the details of the occurrence of an event referenced in paragraph a; (2) provide the facts surrounding that event; and (3) provide the impact such event will have on the project being funded by this award.

c. Upon the occurrence of any of the five events described in the first paragraph, the Government reserves the right to conduct a review of this award to determine the recipient's compliance with the required elements of the award (including such items as cost share, progress towards technical project objectives, and submission of required reports). If the USAMRAA Grants Officer's review determines that there are significant deficiencies or concerns with the recipient's performance under the award, the Government reserves the right to impose additional requirements, as needed, including (1) change the payment method; (2) institute payment controls, and (3) require additional reporting requirements.

d. Failure of the recipient to comply with this term may be considered a material failure by the recipient to comply with the terms of this award and may result in termination.

PROHIBITION OF USE OF LABORATORY ANIMALS**** PROHIBITION – READ FURTHER FOR DETAILS ****

Notwithstanding any other terms and conditions contained in this award or incorporated by reference herein, the recipient is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the USAMRMC, Animal Care and Use Review Office (ACURO). Written authorization to begin research under the applicable protocol(s) proposed for this award will be issued in the form of an approval letter from the USAMRMC ACURO to the recipient. Furthermore, modifications to already approved protocols require approval by ACURO prior to implementation. For each fiscal year, the recipient shall maintain, and upon request from ACURO, submit animal usage information.

Non-compliance with any of these terms and conditions may result in withholding of funds and/or the termination of the award.

PROHIBITION OF USE OF HUMAN SUBJECTS**** PROHIBITION – READ FURTHER FOR DETAILS ****

Research under this award involving the use of human subjects, to include the use of human anatomical substances or identifiable private information, shall not begin until the USAMRMC's Office of Research Protections (ORP) provides authorization that the research may proceed. Written approval to begin research will be issued from the USAMRMC ORP, under separate notification to the recipient. Written approval from the USAMRMC ORP is also required for any subrecipient that will use funds from this award to conduct research involving human subjects.

Research involving human subjects shall be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP. Complete study records shall be maintained for each human research study and shall be

made available for review by representatives of the USAMRMC. Research records shall be stored in a confidential manner so as to protect the confidentiality of subject information.

The recipient is required to adhere to the following reporting requirements:

Submission of major modifications to the protocol, continuing review documentation, and the final report are required as outlined in the USAMRMC ORP approval memorandum.

Unanticipated problems involving risks to subjects or others, subject deaths related to participation in the research, clinical holds (voluntary or involuntary), and suspension or termination of this research by the IRB, the institution, the Sponsor, or regulatory agencies, shall be promptly reported to the USAMRMC ORP.

The knowledge of any pending compliance inspection/visits by the FDA, ORP, or other government agency concerning this clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions, and any instances of serious or continuing noncompliance with regulatory requirements that relate to this clinical investigation or research, shall be reported immediately to the USAMRMC ORP.

Non-compliance with these terms and conditions may result in withholding of funds and/or the termination of the award.

PROHIBITION OF USE OF HUMAN CADAVERS

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Research under this award using human cadavers for testing military equipment or military protective gear shall not begin until the USAMRMC's ORP approves the protocol. Written approval to begin research or subcontract/subgrant for the use of human cadavers under the applicable protocol proposed for this award will be issued from the USAMRMC ORP under separate notification to the recipient.

Non-compliance with these terms and conditions may result in withholding of funds and/or the termination of the award.

PATENTS AND INVENTIONS REPORTING REQUIREMENTS

a. iEdison and annual reporting. The recipient shall electronically file Invention Disclosures and Patent Applications using the Interagency Edison (iEdison) system through the National Institutes of Health (<https://s-edison.info.nih.gov/iEdison>) within the times specified for reporting. In addition, inventions made during the year shall also be reported annually (within 30 days of the anniversary date of the award) on a DD Form 882, "Report of Inventions and Subcontracts." The report shall be sent electronically to USAMRAA.Green@amedd.army.mil. If there are no inventions during the year, no annual DD Form 882 is required. The DD Form 882 can be accessed at <https://www.usamraa.army.mil>.

b. Closeout report. A final DD Form 882 is required. The form shall be submitted electronically to USAMRAAcloseout@amedd.army.mil within 90 days of end of the term of award and shall list all inventions made during the term of the award, or state "none", as applicable. The award will NOT be closed until all reporting requirements have been met.

FINANCIAL REPORTING REQUIREMENTS

The recipient shall use the Standard Form (SF) 425, "Federal Financial Report," for reporting individual awards. Quarterly and final reports are required for those awards receiving advance payments. Annual and final reports are required for those awards receiving cost reimbursement payments.

The Federal Financial Reporting period end dates fall on the end of the calendar quarter for quarterly reports (3/31, 6/30, 9/30, 12/31), end of the calendar year for annual reports (12/31), and the end date of the term of award for the final report. Reports are due 30 days after the reporting period end date for quarterly and annual reports and 90 days after the end date of the term of the award for the final report.

Submission Instructions:

a. Access the SF425 form and instructions through the web site <https://usamraa.army.mil>. Complete the form on this site and select the "Submit form" button. An email will be automatically generated to submit to the address USAMRAASF425@amedd.army.mil. Before sending the email, change the subject line to read the award number assigned by USAMRAA, which is W81XWH-12-1-0011.

b. A separate email must be submitted for each award. DO NOT include reports for multiple awards in the same email. All SF425s shall be submitted electronically to USAMRAASF425@amedd.army.mil utilizing the procedure outlined above.

TECHNICAL REPORTING REQUIREMENTS

a. Annual and Final Technical Reports

Annual and final technical reports are required. Submission of annual and final technical reports, in electronic format (PDF or Word file only), shall be submitted to <https://ers.amedd.army.mil>. Problems accessing this site should be brought to the attention of the USAMRMC Help Desk at 301-619-2049.

Annual reports shall provide a complete summary of the research accomplishments to date with respect to the approved SOW. Journal articles can be substituted for detailed descriptions of specific aspects of the research, but the original articles shall be attached to the report as an appendix and appropriately referenced in the text. The importance of the report to decisions relating to continued support of the research can not be over-emphasized. An annual report shall be submitted within 30 calendar days of the anniversary date of the award for the preceding 12 month period. If the award period of performance is extended by the USAMRAA Grants Officer, an annual report is still required to be submitted within 30 days of the anniversary date of the award.

A final report summarizing the entire research effort, citing data in the annual reports and appended publications, shall be submitted within 90 days of the end of the term of award. The final report shall provide a complete reporting of the research findings. Journal publications can be substituted for detailed descriptions of specific aspects of the research, but an original copy of each publication shall be attached as an appendix and appropriately referenced in the text. The final report shall include a bibliography of all publications and meeting abstracts and a list of personnel receiving pay (do not include salaries/stipends) from the research effort.

b. Format Requirements

Format requirements are provided at https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting.

Although there is no page limitation for the reports, each report shall be of sufficient length to provide a thorough description of the accomplishments with respect to the approved SOW. All reports shall have the following elements, in this order:

FRONT COVER: The Accession Document (AD) Number should remain blank.

STANDARD FORM (SF) 298 "Report Documentation Page:" The abstract in Block 14 shall state the purpose, scope, and major findings, and be an up-to-date report of the progress in terms of results and significance. Subject terms are keywords that may have been assigned to the abstract or are keywords that may be significant to the research. The number of pages shall include all pages that have printed data (including the

front cover, SF298, table of contents, and all appendices). Count pages carefully to ensure legibility and that there are no missing pages, as this delays processing of reports. Page numbers shall be typed.

TABLE OF CONTENTS: The text of the report shall include all sections addressed in the table of contents, to include:

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose, and scope of the research.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved SOW. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but shall be referenced in the body of the report. If applicable, for each task outlined in the SOW, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included. **However, changes to the original SOW shall be approved by the USAMRAA Grants Officer through an award modification prior to initiating any changes.**

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research, to include:

- manuscripts, abstracts, presentations;
- patents and licenses applied for and/or issued;
- degrees obtained that are supported by this award;
- development of cell lines, tissue, or serum repositories;
- informatics such as databases and animal models, etc.;
- funding applied for based on work supported by this award;
- employment or research opportunities applied for and/or received based on experience/training supported by this award.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and, when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e., format used in Science, Military Medicine, etc.).

APPENDICES: Attach all appendices that contain information that supplements, clarifies, or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, surveys, etc.

SUPPORTING DATA: All figures and/or tables shall include legends and be clearly marked with figure/table numbers.

Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.**

Mark all pages of the report which contain proprietary or unpublished data that should be protected by the U.S. Government. REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE. It is the responsibility of the PI to advise the USAMRMC when restricted limitation assigned to a document can be downgraded to Approved for Public Release. DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS.

MANUSCRIPTS/REPRINTS

Copies of manuscripts or subsequent reprints resulting from the research shall be submitted to the GOR at cdmrp.reporting@amedd.army.mil.

DELINQUENT REPORTS

If the recipient is delinquent on reporting requirements for other USAMRAA-sponsored awards, payments on this award may be withheld until acceptable delinquent reports have been submitted.

INVOICING FOR ADVANCE PAYMENTS WITH FULL FUNDING (JANUARY 2012)

a. Payments. Advance payments will be made to the recipient upon receipt of an invoice submitted through Wide Area Work Flow (WAWF) in accordance with the Contract Line Item Number (CLIN) structure set forth in this award. It is anticipated that Defense Finance and Accounting Service (DFAS) will disburse funds within 30 days of receipt of a proper invoice.

b. **A copy of the most recently submitted Federal Financial Report (SF 425) shall be attached in WAWF and submitted with each invoice for all invoice submissions subsequent to the initial invoice submission.**

c. Electronic Funds Transfer (EFT). All payments will be made by EFT to the recipient's financial institution account listed in the Central Contractor Registry (CCR) (located at <https://www.bpn.gov/ccr>). Failure to update CCR will result in nonpayment.

d. If the recipient fails to perform or if the WAWF invoice submission does not have the most recent SF425 attached, the invoice will be rejected.

e. Interest Bearing Account. Unless exempted by applicable Treasury-State agreements in accordance with the Cash Management Improvement Act (CMIA) (31 U.S.C. 3335), the recipient shall deposit all advance payments into an interest bearing account. Interest over the amount of \$250 per year shall be remitted annually to the U.S. Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, Maryland 20852. A copy of the transmittal letter stating the amount of interest remitted shall be sent electronically to USAMRAA.Green@amedd.army.mil.

f. Invoicing Schedule for Advances

Year One \$107,963

<u>Amount</u>	<u>Invoice Submission Date (on or about)</u>	<u>CLIN</u>
\$53,982	Upon execution of this award	(CLIN #0001)
\$17,994	15 August 2012	(CLIN #0001)
\$17,994	15 November 2012	(CLIN #0001)
\$17,993	15 February 2013	(CLIN #0001)

ELECTRONIC INVOICING INSTRUCTIONS (JANUARY 2012)

Wide Area Work Flow (WAWF) is the required method to electronically process recipient request for payment. WAWF allows DOD recipients to submit and track invoices electronically.

Recipients shall (i) register to use WAWF at <https://wawf.eb.mil> and (ii) ensure an electronic business point of contact (POC) is designated in the Central Contractor Registration site at <http://www.ccr.gov> within ten (10) calendar days after award of this Assistance Agreement.

Questions concerning specific payments should be directed to the Defense Finance and Accounting Service (DFAS) Rome, NY at 1-800-553-0527. You can also access payment and receipt information using the DFAS web site at <http://www.dfas.mil/dfas/contractorsvendors.html>. The award number or invoice number will be required to inquire about the status of your payment.

The following codes and information are required to initiate the invoice and assure successful flow of WAWF documents.

TYPE OF DOCUMENT: Grant and Cooperative Agreement Voucher

CAGE CODE: **0V3R3**

ISSUE BY DODAAC: **W81XWH**

ADMIN BY DODAAC: **W81XWH**

INSPECT BY DODAAC: **W81XWH**

ACCEPT BY DODAAC: **W81XWH**

SHIP TO DODAAC: **W81XWH**

LOCAL PROCESSING OFFICE DODDAC: **Not Applicable**

PAYMENT OFFICE FISCAL STATION CODE: **HQ0302**

EMAIL POINTS OF CONTACT LISTING:

INSPECTOR: **USAMRAA.Green@amedd.army.mil**

ACCEPTOR: **USAMRAA.Green@amedd.army.mil**

RECEIVING OFFICE POC: **USAMRAA.Green@amedd.army.mil**

CONTRACT ADMINISTRATOR: **Leave Blank**

CONTRACTING OFFICER: **Leave Blank**

ADDITIONAL CONTACT: **Michelle.Wilhide@amedd.army.mil**

For more information contact **Michelle Wilhide 301-619-4024**

AWARD CLOSE OUT

a. The following documents shall be submitted within 90 calendar days of the end of the term of the award:

(1) Final SF425, "Federal Financial Report" (form available on web site <https://www.usamraa.army.mil>)

(2) Final Technical Report

(3) Final DD Form 882, "Report of Inventions and Subcontracts" (form available on web site <https://www.usamraa.army.mil>)

(4) Cumulative listing of only the nonexpendable personal property acquired with award funds for which title has not been vested to the recipient, if applicable. (This may be submitted on institution letterhead.)

(5) "Volunteer Registry Data Sheet," USAMRDC Form 60-R, if applicable. (Form available on the USAMRMC ORP web site <https://www.usamraa.army.mil/pages/pdf/60r.pdf>.) The PI shall complete a form for each subject enrolled in this study and forward in accordance with ORP requirements.

b. In the event a final audit has not been performed prior to the closeout of the award, the sponsoring agency retains the right to recover an appropriate amount after fully considering the recommendations on disallowed costs resulting from the final audit.

c. The recipient shall promptly refund any unspent balances of funds the DOD Component has advanced or paid that is not authorized to be retained by the recipient. **Make check payable to the U.S. Treasury and mail to:**

USAMRAA
Attn: MCMR-AAA-S
Award No. W81XWH-12-1-0011
820 Chandler Street
Fort Detrick, Maryland 21702-5014

(End of Summary of Changes)